21* Genotypes and borderline sweat tests in Irish CF patients – an update

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The sweat test (ST) is regarded as the gold standard diagnostic test for cystic fibrosis (CF). Borderline STs (i.e. 40–60 mmol/L) present particular difficulties, not only in the clinical diagnosis of a patient, but also in terms of genetic counselling. Borderline results are seen in 4% of STs; 23% of these patients will subsequently be found to have two CFTR mutations, one of which is usually "mild". As part of a data audit required in order to maintain testing and reporting standards, we correlated CFTR mutations in 124 patients referred to the NCMG with "borderline" or "equivocal" STs, over a 12 year period from 1995 to 2007 inclusive. If not originally provided, ST levels were sought retrospectively for the purpose of the audit. ST levels were obtained for 96/124 (77%) of referrals. 39% (37/96) of referrals with "borderline" STs actually had normal ST levels according to accepted reference ranges. None of the referrals with normal STs had two CFTR mutations. 51% of referrals (49/96) were confirmed to have borderline ST levels. Of these, 82% (40/49) had no mutation, 12% (6/49) had one classic mutation and 6% (3/49) had 2 mutations (one classic and one mild). 10% (9/77) had ST levels in the diagnostic range. The high proportion of referred "borderline" STs that actually had normal levels indicates a problem with interpretation or use of ST guidelines by clinicians. The implications for genetic testing services are: (1) data audit is required in order to maintain testing and reporting standards (2) ST results should be sought if not provided with a query CF referral (3) ST results should be obtained before performing comprehensive mutation screening.

22 Genotype–phenotype correlation in Russian CF patients

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Clinical features of two groups of Russian CF patients according to their genotypes were analyzed. The group with severe genotypes consists of 257 patients with two mutations of I and/or II classes, the second group with mild genotypes consists of 23 patients with at least one mutation of IV and/or V classes. The disease began more frequent from digestion abnormalities among patients with severe genotypes, and from bronchopulmonary complications among patients with mild genotypes ($p < 0.01$). The age of diagnosis was significantly higher in mild genotyped patients than in severe genotyped patients ($p < 0.001$). The difference between the mean age of the first disease symptoms and the mean age of diagnosis was larger in the group with mild genotypes ($p < 0.001$). The age of onset of the intestinal disease symptoms was lower in severe genotyped patients than in mild genotyped patients ($p < 0.05$). Severe progression of disease was at lower age in the group with two mutations of I and/or II classes ($p < 0.001$). The decrease of nutrition status ($p < 0.01$), the deterioration of pulmonary function (FVD<70%) ($p < 0.025$) and bronchial colonisation with *P.aeruginosa* ($p < 0.001$) were diagnosed significantly earlier in patients with severe genotypes. Intestinal and hepatobiliary abnormalities were more rare among patients with at least one mild mutation ($p < 0.001$). Meconium ileus and distal intestinal obstructive syndrome were diagnosed only among patients with severe genotypes. Liver disease (fibrosis and cirrhosis) were more frequent in severe genotyped patients than in the mild genotyped group ($p = 0.056$). The mean age of patients with liver disease was lower in the group with severe genotypes ($p = 0.099$).
Supported by: Russian Found of Fundamental Researches (07–04–00090; 05–04–48135).

23 CF gene large deletions: possible genotype/phenotype association

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Aim: Recently new methodologies allow to identify mutations for large deletions in CF alleles (CFTRdel) and to date the estimated frequency is 1–3%. In our Center CFTRdel account for 4.2%of CF mutations. The aim of this study is to investigate the possible genotype–phenotype association.
Methods: 490 alleles of 245 CF patients have been studied. Alleles not identified by conventional methods (PCR OLA–DHPLC sequency) were studied with the MLPA technique (SALSA CFTR MLPA kit, MRC Holland) in order to detect deletions of entire exons. Clinical data-pancreas status, *Pseudomonas* (Ps) infection, clinical outcome-of patients carrying CFTRdel were examined.
Results: We identified CFTRdel in 21alleles, characterizing 20 CF patients, mean age19.5 years (0.5–40).
Three different deletion types were found: CFTRdel22–23–24 (11/21 alleles), CFTRdele2 (7/21 alleles), CFTRdele14b-17b, and CFTRdele 2–3.
Mutations carried on the other allele were F508del (12/20), G542X (2/20), L1065P, G1244E, 2789+5G>A, R347P. Two patients presented private mutations (F311del and S737F); one was homozygous CFTRdel22–23–24.
Except for two with private mutations, all patients presented pancreatic insufficiency (90%). PS chronic infection was present in 15/20 (75%), and in 10/12 (83%) F508del heterozygous. Ps first isolation was precocious (mean age 3.4 y, range 0.5–11).
Four patients underwent a double lung transplantation (mean age 30 y) and two died in waiting list (mean age 20 y) five of them were compound heterozygous for F505del.
Conclusion: To date little is known about the effect of CFTRdel. We report clinical features in 20 CF patients: most of them had pancreas insufficiency, early Ps colonisation, chronic Ps infection, poor clinical outcome. These data seem to suggest that CFTRdel correlate with severe phenotype.

24* Occurrence of CFTR de novo mutations is not so rare

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Comprehensive CFTR gene studies allow identifying both CF alleles in more than 98% of CF patients. De novo mutations are exceptional, but have been reported. In order to assess their frequency, data from three French reference laboratories (Brest, Creteil, Montpellier) were collected: 2250 families including CF patients with two identified mutations and their parents, i.e. 4500 chromosomes.
Four cases of de novo mutations, all occurring on the paternal chromosome, were documented by analysis of microsatellite markers located within the CFTR gene and on various chromosomes: (1) E116K, in trans of F508del in a 21 y old woman having DB, Pa colonization and positive ST; (2) 296+1G>T, in trans of 3272–26A>G A, in a 10 y old girl with severe ENT manifestations and positive ST; (3) 3200–3204delTAGTG, in trans of F508del, in a girl diagnosed through NBS (positive IRT and ST); (4) a partial duplication of exons 7 and 8 in trans of F508del in a boy detected through NBS and identified by cDNA analysis.
While E116K was already reported in another CF patient, the three other mutations were never described.
In conclusion, this study allowed estimating the frequency of CFTR de novo mutations to 1 in 1100 chromosomes. These data are important for genetic counselling and reflect the need to carry on systematic family studies, especially when private mutations are identified or in cases of fetal hyperechogenic bowel during pregnancy where study of foetal DNA should be recommended.